

Developmental Neurotoxicity of Domoic Acid: Evidence for a Critical Window of Exposure

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Harmful algal blooms are a growing worldwide problem.¹ Toxins produced by some of these algae, including the neurotoxin domoic acid (DomA), may reach humans through contaminated seafood consumption.^{2,3} Because acute high-level exposure may cause amnesic shellfish poisoning,⁴ countries around the world limit DomA to 20 µg/g of shellfish tissue.^{5,6,7} However, relatively little is known about the health effects of chronic low-level exposure such as that experienced by people who regularly eat shellfish.^{8,9,10} In a recent study¹¹ in *Environmental Health Perspectives* investigators based at the Woods Hole Oceanographic Institution (WHOI) in Massachusetts analyzed the developmental neurotoxic effects of DomA in zebrafish to help fill this gap.

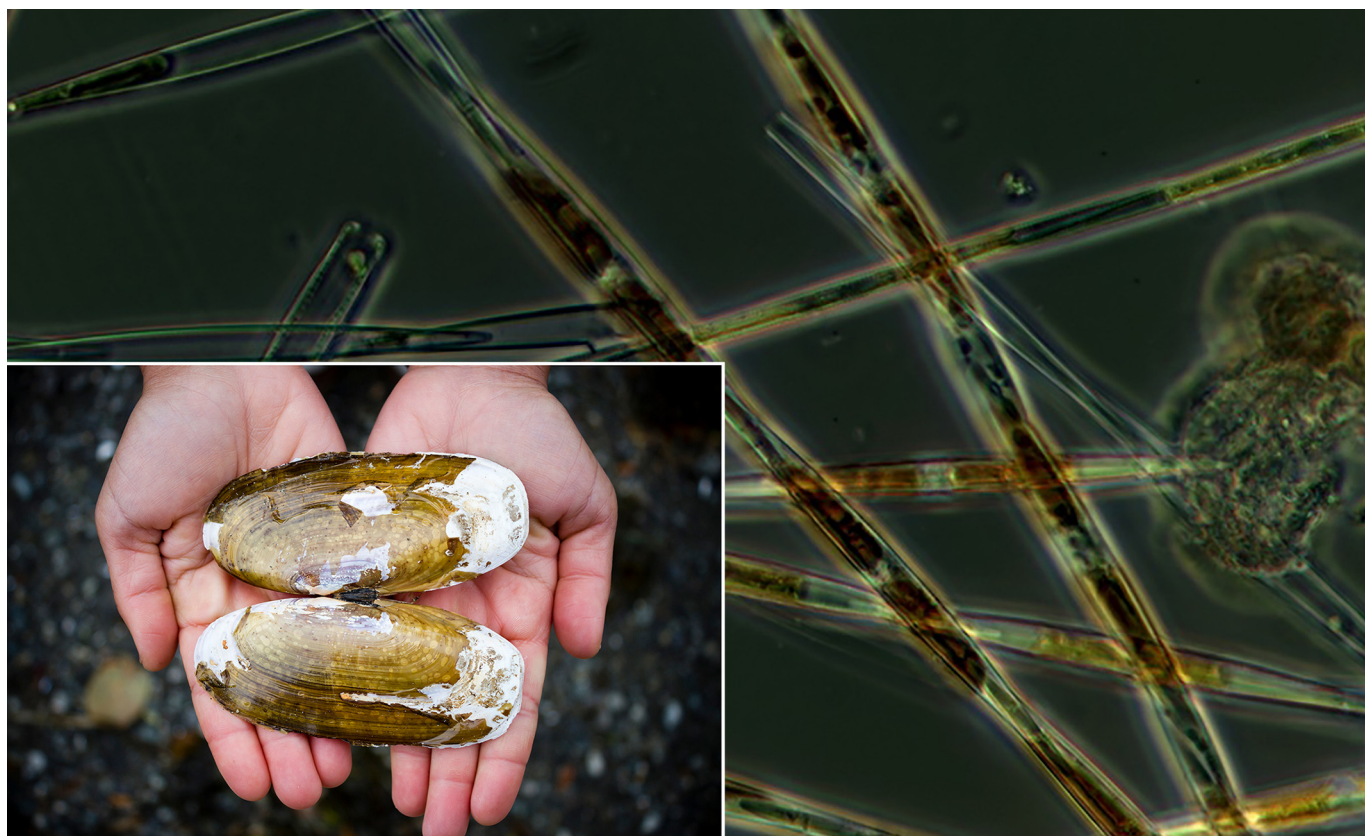
The researchers exposed zebrafish embryos and larvae to DomA doses that were 3- to 260-fold lower than exposures tested in earlier studies.^{12,13} Even the lowest nominal dose of 0.09 ng during a defined developmental window caused behavioral deficits in the larvae. The researchers causally linked these deficits to disrupted myelination processes and altered gene expression.

Zebrafish have distinct advantages as a model organism. Embryos are transparent during early development, and their nervous system structures are similar to those of humans. However, in

zebrafish these structures develop externally rather than hidden inside a uterus. Thus, real-time imaging can reveal changes in labeled cells of interest during very early stages of development.

Instead of the usual method of adding the agent of interest to the fish tanks, the researchers used microinjection into a cardinal vein to deliver a single dose of 0.09–0.18 ng of DomA to the embryos and larvae. They administered doses at specific developmental periods between 1 and 4 days postfertilization (dpf). “Microinjection ensured that the desired dose reached the embryo and let us precisely time exposures throughout development to home in on a critical window,” says first author Jennifer M. Panlilio, who performed the research as a doctoral student in a joint program between WHOI and Massachusetts Institute of Technology.

Following the injection, fluorescence time-lapse microscopy was used to track the movement of specialized cells in the spinal cord and the formation of protective myelin sheaths around axons, the part of the neuron that transmits electrical signals. Larval RNA was sequenced at 3 and 7 dpf, and myelin structure was assessed at 5–7 dpf. At 7 dpf, the researchers measured the larvae’s startle behavior in response to acoustic/vibrational stimuli. Well-known neural circuits and cell types drive this behavior.^{14,15}



Domoic acid is produced by algal species including members of the *Pseudo-nitzschia* genus (shown). It causes amnesic shellfish poisoning, a potentially fatal illness that can strike people who eat contaminated seafood, such as clams, mussels, and crab. The disease was only discovered in 1987.³ Image: *Pseudo-nitzschia*: Vera Trainer/NOAA; razor clams: © iStockphoto/jack looney.

Exposure to 0.09 ng DomA at 2 dpf had effects that were not observed at 1 or 4 dpf. It reduced the expression of genes required for maintaining axon and myelin structure, it produced structural deficits in myelin sheaths, and it delayed and changed typical motion features of the larvae's startle response. However, it had no appreciable effects on mortality or gross morphology.

"Our novel finding is a narrow critical window of development when DomA exposure disrupts the initial myelination of axons," says Panlilio. "This is a potential molecular basis for an observable behavior, which provides an important functional end point for future research." Even if the end point is similar in other organisms, she adds, the critical window may be different. The myelination process in humans, for example, starts *in utero* and continues throughout adolescence.

For Rebekah Petroff, who was not involved in the new study, the results are consistent with observations in rodents,^{16,17,18,19} marine mammals,^{20,21} and nonhuman primates.^{22,23} Petroff, a postdoctoral fellow at the University of Michigan, has studied DomA neurotoxicity in adult crab-eating macaques after low-level exposure.

"Disrupted myelination pathways are a plausible mechanism for developmental end points that have been observed consistently across species," says Petroff. "However, it will be difficult to translate how important these effects are until we know more about human exposure levels." For example, the DomA exposure of fetuses and infants whose mothers consume contaminated shellfish is currently unknown.

Jennifer Freeman, an associate professor of toxicology at Purdue University, appreciates the study's precise targeting of different developmental stages. "I think we need to do more of that in developmental toxicology," says Freeman, who also was not involved in the project. "If you don't capture the susceptible period, you may completely miss an adverse health outcome."

Freeman finds the alignment of multiple pieces of evidence for the critical window of 2 dpf—namely, structural imaging, gene expression analysis, and functional outcome—compelling and considers it critical for regulating other environmental chemicals.

Although researchers have identified several algal genes that produce DomA,²⁴ we have only a limited understanding of the environmental stressors that trigger production of the toxin.²⁵ Rising sea surface temperatures are predicted to increase the frequency of harmful algal blooms, including those with DomA-producing *Pseudo-nitzschia* species.²⁶ DomA may persist in shellfish tissue long after the blooms dissipate, although substantial between- and within-species variation complicates predictions.²⁷ "It's a complex problem that's challenging but important to regulate," concludes Petroff.

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